after intervening storage in phosphate buffer containing bovine serum albumin. Not only was the detection limit for ricin improved by day 4 from 1 µg/L to 0.5 µg/L, but also intraassay data, including well-to-well variation, was improved.

Other important points in immunoassay development are the issues of reliability and stability of stored microplates. The storage time (shelf life) of antigen- or antibody-labeled microplates can be improved by adding stabilizers. Several products on the market today protect the antibody or antigen on the microwell surface during long-term storage, whether wet or dry. Some of these products rely on the protection of immobilized antibody or antigen by inclusion of sugars and bovine serum albumin during the storage or drying process. We have had success with using sucrose as a stabilizer, both in standard microplate formats (4) and in Immobilon AV plates (4). We previously reported a significant increase in ELISA sensitivity for plates stored for several days with capture antibody immobilized on the microplate surface in solutions containing sucrose: Alkaline phosphatase-labeled secondary antibodies to immobilized IgGs had increased sensitivity after microplate storage in buffer containing sucrose (5). Orientation of the immobilized capture antibodies is considered important for recognition by secondary antibody or for capture of antigen. Advantageous reorientation is most likely the case in the deliberately stored plates, which have had time for some antibodies to reorient themselves, or for poorly attached and cross-linked capture antibody to have been removed during chaotroping. These phenomena may result in increased binding by the antigen or secondary antibody (4, 5).

We hope this will help the reader understand that there is still help on the horizon for the researcher using microplate ELISA as a format for immunoassay.

We acknowledge Mark Poli, US Army Medical Research Institute for Infectious Diseases (USAMRIID), Fort Detrick, Frederick, MD, for supply of antibodies to brevetoxicin and ricin used in this research and the Departments of Defense and Commerce SBIR for funding of this research. We also thank Millipore Corp. for the supply of Immobilon AV microplates.

References

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is Bilirubin Protective Against Coronary Artery Disease?

To the Editor:

Schwartzner et al. report a statistically significant inverse correlation between serum total bilirubin concentration and severity of ischemic heart disease (1). They proposed that this may reflect a protective antioxidant role of bilirubin. The bilirubin concentrations they found were within the reference range.

Peripheral vascular disease (PVD), a state of widespread vascular pathology, could also have an effect on the serum bilirubin concentration. We therefore reviewed the biochemical profile of 36 patients with PVD seen in our newly established joint vascular risk-factor clinic. Five patients were excluded because of abnormal results for liver enzymes (increased y-glutamyltransferase or transaminases). The remaining 31 patients (12 women and 19 men) had a median bilirubin concentration of 6 µmol/L (range 3-12) (Table 1). Compared with the reference range in our laboratory, 5-17 µmol/L (mean = 11, SD = 3), this median was considerably >1 SD from the mean reference value. Using a t-test for difference of sample mean from population mean, we found that the bilirubin values in our PVD patients (mean 6.8, SD 2.5 µmol/L) were significantly lower (P < 0.001) than those expected in a healthy population (Table 1).

These results are consistent with a decrease in bilirubin attributable to an exhaustion of other antioxidant systems (e.g., vitamins C and E) - a possibility that can be tested by measuring bilirubin in persons taking antioxidant supplements. The results are also in agreement with Benaron and Bowen (2), who found a significantly lower increase in mean bilirubin concentration in neonates who had illnesses considered to enhance production of free radicals. They proposed that this difference reflected greater scavenging of free reactive oxygen species by bilirubin.

The disorder Gilbert syndrome is characterized by increased serum bilirubin, the increase being amplified by stress (3, 4). If high bilirubin concentrations are protective per se, one would expect to see a lower incidence of ischemic heart disease in Gilbert syndrome. A limited search of the literature reveals that this syndrome, which may affect as many as 5% of the population, is considered a benign condition with a normal life span; however, no attention was drawn to particular causes of death (3, 4). Studies of neonates and Gilbert syndrome, whose hyperbilirubinemia is mainly from unconjugated bilirubin, may

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Table 1. Serum bilirubin in patients with peripheral vascular disease.

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<thead>
<tr>
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<th>n</th>
<th>Median</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
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</thead>
<tbody>
<tr>
<td>Women</td>
<td>12</td>
<td>5.5</td>
<td>3-12</td>
<td>6.2±2</td>
<td>2.4</td>
</tr>
<tr>
<td>Men</td>
<td>19</td>
<td>6.0</td>
<td>3-11</td>
<td>6.9±2</td>
<td>2.5</td>
</tr>
<tr>
<td>All</td>
<td>31</td>
<td>6.0</td>
<td>3-12</td>
<td>6.6±2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Statistical analysis (t-test):
* Not significantly different from PVD men (P = 0.44).
o Significantly different from reference controls (P < 0.001, 2-tailed).
shed light on which fraction expresses the proposed protective role of bilirubin.

References

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Standardization and Iron Assays

To the Editor:

With much interest we read the article of Tietz et al. (1) on the status of serum iron measurements as well as the accompanying editorial by Eckfeldt and Witte (2). In an elegant study of the present state-of-the-art of iron methodology, Tietz et al. applied various routine methods in combination with three reference procedures, while Eckfeldt and Witte questioned in a well-written and provocative way the value of iron measurements. However, Eckfeldt et al. cited us incorrectly. We did not describe in our cited publication (3) the lack of comparability of clinical iron methods; we only criticized the various proficiency testing systems in relation to serum iron.

In describing the clinical iron status, the measurement of serum iron is clearly less important nowadays than it was 20–30 years ago because of the introduction of techniques for determining, e.g., transferrin, ferritin. However, for certain patient groups (e.g., those with iron-deficiency anemia, iron overload, some infectious diseases), serum iron is still a useful marker, especially in combination with transferrin [and not total iron binding capacity (4)]. Assays for iron, especially, but also transferrin, are easy to perform and less expensive than assays for ferritin. Of course, the diurnal variation of iron has to be taken into account.

Thus, the efforts of Tietz et al. deserve appreciation. We support their plea for developing a sound reference method for serum iron so that we can judge the analytical accuracy of iron methods in current use. However, the same consideration applies for all routine methods of proven clinical value: Thorough evaluations are needed according to the hierarchy of the methods. For transferrin and ferritin, standardization procedures are needed to reduce the large analytical variations seen in quality assurance programs.

References

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An author of the editorial cited responds:

To the Editor:

I apologize if I incorrectly cited Blijenberg et al. (1) on serum iron methods and proficiency testing. Although these authors may not have explicitly criticized clinical iron methods, their scattergram comparing the results of a widely used clinical method with those of the 1978 ISCH Reference Method for iron as performed in their laboratory shows a significant systematic bias and scatter of points about the regression line. They also present proficiency testing results that suggest significant systematic bias between several clinical methods and the 1978 ISCH Reference Method. To me, both pieces of data suggest a lack of comparability of clinical iron methods with reference methods, indicating that many iron methods have accuracy problems.

The two main points Witte and I were trying to make in our editorial were: (a) lacking something approaching a “definitive” iron method for serum, it is difficult to say much about the true accuracy of any of the currently available clinical (or reference) methods; and (b) because we believe sorting all this out will take considerable effort, we think laboratorians should be sure that significantly improved clinical utility of the serum iron will actually result. Probably some sort of combination of isotope dilution–mass spectrometry method coupled with a way to separate the various forms of iron (e.g., transferrin-bound iron, hemoglobin-bound, other protein-bound, dextran-bound, etc.) in normal and abnormal serum specimens, proficiency testing fluids, and serum-based calibrator materials will answer the first question. I do not believe that developing additional dye-binding methods will really answer the primary question at hand: When different analytical methods (clinical or reference methods) give systematically different iron results for a given specimen, which method is “right” and how does one define “right”?

As stated previously, Witte and I wanted to stimulate public discussion with our editorial. We join Blijenberg and van Eijk, as well as Tietz and colleagues, in encouraging those with new ideas on the design and validation of clinical, reference, and definitive methods for iron to come forward.

Reference

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